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SOME EFFECTS OF COLLOIDAL METALS ON SPIROGYRA[1]

W. D. HOYT

(WITH FOUR FIGURES)

The effects of colloidal metals on the activities of living cells seem to have received but little attention, although it appears that studies of such effects should yield results of importance to protoplasmic physiology. We regard protoplasm as largely composed of colloidal material, and believe the enzymes affecting many of the protoplasmic processes to be colloidal in their nature. Further, we know that, outside of protoplasm, colloids affect one another in many different ways, and that colloidal metals are capable of bringing about reactions which are similar, in many respects, to those usually related to the action of enzymes. It becomes a matter of considerable interest, therefore, to determine the effects of colloidal metals on protoplasm and protoplasmic products, especially since the soluble salts of many metals are known to be extremely poisonous. The phase of the problem thus suggested with which the present paper has to deal may be stated as follows: Solutions of the salts of silver, gold, and platinum are poisonous to protoplasm; colloidal solutions of the metals named may bring about reactions in non-living materials similar to those caused by enzymes; what, then, may be the effects of such colloidal solutions on protoplasm?

The present investigation was planned to obtain some preliminary information bearing upon the answer to this question. It has mainly to do with the descriptive aspect of some of the superficial effects of colloidal solutions of platinum, gold, and silver on two species of *Spirogyra*, with a few notes on other algae. The work was undertaken at the suggestion of Professor GEORG KLEBS, and was carried out in the Botanical Institute of the University of

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[1] Botanical contribution from the Johns Hopkins University, no. 30. These investigations were carried on while the author held the Adam T. Bruce Fellowship of the Johns Hopkins University. A summary of the results was presented before the Botanical Society of America at its Washington meeting, December 28, 1911.

Heidelberg. Unless otherwise stated, the species used was *Spirogyra longata* (Vauch.) Kg. The material and general methods were the same as those described in a previous paper (**8**). The original culture of *Spirogyra longata* was brought from Algiers, but the alga had been growing for nearly three years in the laboratory, in a stock culture which received small additions of tap water from time to time. The filaments of this stock culture were apparently healthy and of the usual appearance. The colloidal solutions of silver, gold, and platinum here employed were made with non-toxic water, according to the method previously described, of distillation in glass with animal charcoal present in the retort. They were kindly prepared for the author by Dr. W. FRAENKEL in the laboratory of Professor G. BREDIG at the University of Heidelberg, by atomizing the metals with an electric arc under water. This method has been described by BREDIG (**2, 3, 4**). The solutions were kept in flasks of Jena glass. Those of gold and platinum were determined by Dr. FRAENKEL and found to contain 90 ppm. (parts per million) of gold and 96 ppm. of platinum, respectively. The sample of the silver solution was unfortunately lost before being determined, but, as all three solutions were originally prepared so as to contain the same amounts of metal (with an error not greater than 10 ppm.), the concentration of the silver solution here employed may be considered as approximately 90 ppm. The solution of gold was purple in color, that of platinum was yellow-brown, while the solution of silver was grayish brown. The gold solution, on standing, formed a deposit, probably of the larger particles, on the walls both of the flask containing the stock solution and of the culture dishes, but the intensity of its color was not perceptibly diminished by this deposition. The solutions of silver and platinum gave no observable deposits. In considering the results obtained with these solutions, the possibility is not to be forgotten that small amounts of metal oxides may have been formed in the process of preparation, and that the solutions are not to be considered as necessarily and entirely free from metal in the ionic condition.

Colloidal solutions of silver, gold, and platinum prepared by BREDIG, similar to those used by the author except that they were weaker and were not made with carbon-distilled water, were examined by ZSIGMONDY (17) with the ultra-microscopic apparatus devised by him. From his results it was calculated that the diameters of the particles in the silver solution were about 50–77 $\mu\mu$. The gold solution contained particles 20–80 $\mu\mu$ in diameter, the larger ones being separable by filtration. There was a much greater range in the size of the suspended platinum particles, but by far the greater portion of these were very small and had an average diameter of 44 $\mu\mu$. As the solutions employed in the present investigation resembled in appearance those described and examined by ZSIGMONDY, it is probable that the particles in these solutions had a similar degree of magnitude.

The results upon toxicity obtained in the present investigation are shown in tables I–IV, which are self-explanatory. In the following consideration of the main points the Roman numerals in parentheses refer to the tables and the Arabic ones to the experiment numbers as there given.

The solution of silver was extremely toxic (I, 1), killing the alga within 17 hours in concentrations above 0.045 ppm. (0.05 per cent of the solution originally prepared) and injuring many of the filaments in strengths as low as 0.00225 ppm. (0.0025 per cent of the original solution). In filaments thus killed the cell contents were often dark and disorganized and were more or less contracted.

Various substances were added to the colloidal silver solution to determine the effect of their presence upon its toxicity. The addition of salts to form a 0.5 per cent concentration of Crone's nutrient solution[2] to colloidal solutions of silver in concentrations of 0.045 ppm. or less (I, 2), produced marked improvement, as did also the addition of about 81 ppm. colloidal platinum (I, 3), or of about 0.1 gr. animal charcoal (I, 4). Solutions which had previously been injurious or fatal were thus changed into solutions which were entirely or almost entirely non-injurious during the period of the experiments (1–3 days). In such cases the cell contents retained their usual healthy appearance.

The effect of the gold solution shows that it was much less toxic than that of silver. In order to obtain colloidal gold and hold it in solution, about 0.02 per cent of sodium hydroxide was added to the water with which the colloidal solution was prepared, so that this solution contained NaOH. Two experiments were carried out with *Spirogyra* from the stock culture, filaments being transferred to the undiluted, alkaline solution of colloidal gold (II, 5*a*). In one case the filaments remained in excellent condition during the period of the experiment (2 days); in the other case, the alga remained with-

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[2] This solution contained salts in the following proportions: 1 gr. KNO_3; 0.5 gr. $MgSO_4$; 0.5 gr. $CaSO_4$; 0.25 gr. $Ca_3(PO_4)_2$; 0.25 gr. $Fe_3(PO_4)_2$.

TABLE I

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL SILVER SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Silver in medium, approximately, ppm.	Other substances in medium, approximate amounts	Condition of plants*	Duration of experiment, days*
1*a*	90.0		D, D	1, 1
1*b*	45.0		D	1
1*c*	22.5		D	1
1*d*	4.5		D	1
1*e*	0.9		D	1
1*f*	0.108		D	1
1*g*	0.045		P, D	1, 1
1*h*	0.0135		E	1
1*i*	0.009		Gp	3
1*j*	0.0045		E	1
1*k*	0.00225		G, D	3, 1
2*a*	0.045	Salts to form 0.5 p.c. Crone's solution	E, Eg	2, 1
2*b*	0.0135	*do.*	E	1
2*c*	0.009	*do.*	Eg	3
2*d*	0.0045	*do.*	E	1
3*a*	0.045	Colloid. Pt, 81 ppm.	E, E	2, 1
3*b*	0.0135	*do.*	E	1
3*c*	0.009	*do.*	E	3
3*d*	0.0045	*do.*	E	1
4*a*	0.045	Animal charcoal, 0.1 gr.	E, G	2, 1
4*b*	0.0135	*do.*	E	1
4*c*	0.009	*do.*	E	3
4*d*	0.0045	*do.*	E	1

* In tables I–IV the condition of the plants is indicated as follows: *E, excellent,* meaning that none of the filaments seemed injured; *G, good,* meaning that less than half seemed injured; *P, poor,* meaning that more than half seemed injured; *D, dead,* meaning that all or practically all the filaments were dead. A combination of letters denotes a condition between those denoted by the combined letters, respectively; thus, *Eg* means a condition between *excellent* and *good,* etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the last column (duration in days) refer to the experiments in the same order as do the letters; thus, the first number denoting *duration* and the first letter denoting *condition* both refer to the same experiment, etc.

out apparent injury for 5 days, except that the chloroplasts dwindled slightly; but on the sixth day, when the experiment was discontinued, many filaments were dead. For comparison with the above, two experiments were performed with *Spirogyra* which had grown in 0.1 per cent Crone's solution for 72 days. The filaments were rinsed in non-toxic water and placed in the undiluted gold solution (II, 5*b*). In one case, the filaments remained in excellent condition two days, but a few showed injury on the third day; in the other case, the alga remained in excellent condition one day, but on the

TABLE II

CULTURES OF SPIROGYRA LONGATA IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT COLLOIDAL GOLD AND OTHER ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
5*a*†............	0.02	Colloid. Au, 90.0	E, Eg	2, 6
5*b*............	0.02 [Plants from 0.1 p.c. Crone's sol., 72 days old]	Colloid. Au, 90.0	Eg, P	3, 4
5*c*............	0.01	Colloid. Au, 45.0	Gp	2
6.............	0.02	....	D	1
7.............	0.01	....	Pd	5
8.............	0.01	Colloid. Pt, 48.0	E	5
9*a*............	0.02	Colloid. Ag, 0.0135	D	1
9*b*............	0.02	Colloid. Ag, 0.009	Pd	1
9*c*............	0.02	Colloid. Ag, 0.0045	Gp	1
10.............	0.02	....	Eg, D	1, 1
10*a*............	0.01	....	E, Eg	1, 1
11*a*............	0.02	$AuCl_2$, 100.0	P, D	1, 1
11*b*............	0.01	$AuCl_2$, 100.0	Eg, Gp	1, 1
12*a*............	0.02	Colloid. Pt, 86.4	E, G	1, 1
12*b*............	0.01	Colloid. Pt, 86.4	E, E	1,1

* The sodium hydrate solution used in cultures 5*a*–9*c* was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 10–12*b* was prepared at another time. The strength of the former solution is given only approximately, that of the latter is given accurately.

† Unless otherwise noted, the material of *Spirogyra* used in these experiments was from the stock culture.

TABLE III

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL PLATINUM SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Platinum in medium, approximately, ppm.	Other substances in medium, approximate amounts, per cent	Condition of plants	Duration of experiment, days
13	96.0	....	Eg, E	22, 3
14*a*	86.4	KCl, 0.1	P	12
14*b*	....	*do.*, control to 14*a*	Pd	6
15*a*	86.4	$MgSO_4$, 0.02	Gp	19
15*b*	....	*do.*, control to 15*a*	Pd, D	6, 2
16*a*	48.0	Tap water, 50.0	G	20
16*b*	....	*do.*, control to 16*a*	Pd	12
16*c*	48.0	Ord. dist. H_2O, 50.0	Gp	26
16*d*	....	*do.*, control to 16*c*	D, D, D	2, 1, 1
17	....	$PtCl_4$, 0.008 $MgSO_4$ 0.02	Pd	12

TABLE IV

CULTURES OF SPIROGYRA DECIMINA (NOS. 18–23*b*) AND OF OSCILLATORIA (NO. 24) IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
18	0.02	Colloid. Au, 90.0	Pd	4
19	0.01	....	Pd	2
20	0.01	Colloid. Pt, 48.0	E	2
21*a*	0.02	....	Eg	1
21*b*	0.01	....	E, Eg	1, 1
22*a*	0.02	$AuCl_2$, 100.0	Eg	1
22*b*	0.01	*do.*	Eg, Gp	1, 1
23*a*	0.02	Colloid. Pt, 86.4	E	1
23*b*	0.01	Colloid. Pt, 86.4	E, E	1, 1
24†	0.02	Colloid. Au, 90.0	E, E	4, 3

* The sodium hydrate solution used in cultures 18–20 and 24 was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 21*a*–23*b* was prepared at another time.

† The alga used in no. 24 was *Oscillatoria* from a 0.1 per cent Sachs's solution, thickened with 1 per cent agar.

second and third days many filaments were injured, while on the fourth day practically all were dead.

Diluting the gold solution with an equal volume of non-toxic water produced no improvement. In such a solution *Spirogyra* from the stock culture remained in excellent condition one day, but on the second day about half of the filaments were dead (II, 5*c*).

The effect of transferring portions of *Spirogyra* from colloidal gold solution to non-toxic distilled water was tested. Filaments which had been in gold solution for two days, when placed in this water, showed injury within one day and were, for the most part, dead within two days, while the portions of the alga remaining in the gold solution were still in excellent condition. Other effects of such transfers will be described later.

Although the colloidal gold solution itself was only slightly injurious, the sodium hydrate solution (about 0.02 per cent) used in its preparation was extremely toxic, killing all the filaments and causing contraction of their cell contents within 17 hours (II, 6). This alkaline water, when diluted with an equal volume of non-toxic distilled water, was still decidedly toxic (II, 7), but was not injurious during the period of the experiment (5 days) when diluted with an equal volume of the full strength colloidal platinum solution (II, 8). A similar improvement by the addition of colloidal platinum was produced on 0.02 per cent and 0.01 per cent solutions of NaOH prepared at another time (II, 10, 12). Even weak solutions of silver seemed to produce a beneficial effect on this alkaline solution, since the alga lived better with the addition of 0.01–0.005 per cent of the full strength silver solution (0.009–0.0045 ppm.) than in the NaOH solution alone (II, 9). The addition, however, of 0.01 per cent of $AuCl_2$ to 0.02 per cent and 0.01 per cent solutions of NaOH produced no improvement in these solutions; on the contrary, the soluble gold salt seemed to make the NaOH solutions more injurious (II, 10, 11).

The colloidal solution of platinum was still less injurious than that of gold, filaments of *Spirogyra* placed in this solution remaining in apparently perfect condition for 9 days and dying only after 22 days (III, 13). Not only was this platinum solution not injurious during the first few days of the experiments, but it produced improvement in a 0.1 per cent solution of KCl (III, 14), in a 0.02 per cent solution of $MgSO_4$ (III, 15), and in a mixture with an equal volume of tap water or an equal volume of ordinary distilled water (III, 16), over such solutions without the colloidal platinum. It also, as has been shown above, produced improvement in a weak colloidal solution of silver and in a solution of NaOH. The addition, however, of 0.008 per cent $PtCl_4$ to a 0.02 per cent solution of $MgSO_4$ produced no improvement in this solution (III, 17).

A few experiments were made with other algae. *Spirogyra decimina* Kg.,[3] newly brought to the laboratory, gave practically the same results as those already described, except that it was more quickly injured by colloidal gold solution (IV, 18–23). However, filaments of a species of *Oscillatoria* growing in 0.5 per cent Sachs's solution thickened with 1 per cent agar, when transferred to the gold solution remained in perfect condition and continued their usual movements during the period of the experiment (3–4 days) (IV, 24).

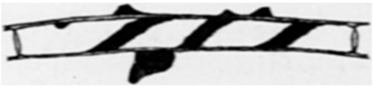

FIG. 1.—*Spirogyra longata* from stock culture, 24 hours after transfer to colloidal gold solution; examined and drawn in gold solution.

When filaments of *Spirogyra longata* from the stock culture or filaments of *S. decimina* were placed in colloidal gold solution, a striking phenomenon was observed; the outer layer of the cell walls swelled irregularly within 17 hours, forming gelatinous sheaths over considerable portions of the external surfaces (fig. 1). These sheaths, whether greatly swollen or not, were colored purple by the gold solution. The cells remained in otherwise excellent condition during this time; their contents were uncontracted and normal in appearance, and the chloroplasts retained their usual green color. That the cells were alive and healthy was indicated by the fact that they were easily plasmolyzed by glycerine. When filaments of *S. longata* were examined directly in the gold solution, they showed slight irregular swellings (fig. 1), but when transferred from the gold solution to glycerine solution or to non-toxic distilled water marked swelling became evident over the entire wall within one or two minutes (figs. 2, 3). Under the latter conditions the modified outer layer frequently became ruptured, breaking away from the wall in purple, gelatinous masses and leaving the remainder of the wall and the other portions of the cell uncolored and apparently unaffected. Filaments of *S. decimina* formed swollen sheaths in the gold solution itself similar to those formed by *S. longata* when transferred from this solution to water. After a sheath had broken from a portion of the wall, no new sheath was formed, the rest of the wall remaining unswollen and uncolored during the period of the experiments (2–6 days). The results of further studies upon the formation of these gelatinous sheaths are presented in table V. Where difficulty was experienced in determining by simple microscopic observation whether or not swelling of the outer wall had occurred, such difficulty was removed by treatment of the material with Bismarck brown, which so stains the unswollen and swollen portions of the walls as to render even very thin sheath layers clearly discernible.

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[3] Determined from O. KIRCHNER, Die mikroskopische Pflanzenwelt des Süsswassers. 1885.

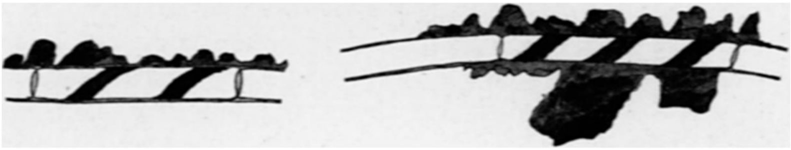

FIG. 2 FIG. 3

FIGS. 2 and 3.—*Spirogyra longata* from same material as that shown in fig. 1, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

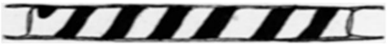

FIG. 4.—*Spirogyra longata* from culture (72 days old) in 0.1 per cent Crone's solution, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

Sheaths were formed in the gold solution by *Spirogyra longata* from the stock culture (V, 1*a*), and by *S. decimina* (V, 1*c*), and they were produced to a very slight degree by a species of *Oscillatoria* (V, 1*d*). No sheaths were thus formed upon filaments of *S. longata* (fig. 4) which had been for 72 days in 0.1 per cent Crone's solution (V, 1*b*), nor by a species of *Hormidium* (V, 1*e*). Sheaths failed to appear upon *S. longata* in colloidal gold solution diluted with an equal volume of non-toxic water (V, 2), nor were they evident upon

TABLE V

VARIOUS TREATMENTS PRODUCING EXTERNAL SHEATHS ON ALGA CELLS, TOGETHER WITH COMPARATIVE TREATMENTS FAILING TO PRODUCE SUCH SHEATHS

CULTURE NO.	ALGA EMPLOYED	MEDIUM EMPLOYED, NON-TOXIC DISTILLED WATER CONTAINING		NUMBER OF TESTS IN WHICH SHEATHS WERE		NOTES*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
1*a*	*Spirogyra longata*†	Gold, 90.0	NaOH, 0.02‡	2	....	E; sheaths formed within 17 hours
1*b*	*Do.*, from culture in 0.1 p.c. Crone's sol. 72 days old	*do.*	*do.*	....	2	G
1*c*	*Spirogyra decimina*§	*do.*	*do.*	1	....	D; within 4 days. Sheaths formed within 17 hours
1*d*	*Oscillatoria*‖	*do.*	*do.*	1 (?)	1	E; a few filaments in one culture showed slight sheaths when transferred to tap water; no sheaths apparent in gold solution. Usual movements observed
1*e*	*Hormidium*	*do.*	*do.*	....	1	....
2	*Spirogyra longata*	Gold, 45.0	NaOH, 0.01‡	....	1	....
3*a*	*Do.*	....	AuCl$_2$, 0.01; NaOH, 0.02¶	....	2	D; many filaments
3*b*	*S. decimina*	....	*do.*	....	1	G
4*a*	*S. longata*	....	AuCl$_2$, 0.01; NaOH, 0.01¶	....	2	G
4*b*	*S. decimina*	....	*do.*	....	2	G
5	*S. longata*	....	NaOH, 0.02‡	....	1	D
6*a*	*Do.*	....	NaOH, 0.01‡	....	1	D
6*b*	*S. decimina*	....	*do.*	1	....	No sheaths after 17 hours, but some colorless ones appeared on second day, when filaments mostly D
7*a*	*S. longata*	....	NaOH, 0.02¶	....	2	G, in one culture D, in other
7*b*	*S. decimina*	....	*do.*	....	1	D
8*a*	*S. longata*	....	NaOH, 0.01¶	....	2	G

TABLE V—*Continued*

CULTURE NO.	ALGA EMPLOYED	MEDIUM EMPLOYED, NON-TOXIC DISTILLED WATER CONTAINING		NUMBER OF TESTS IN WHICH SHEATHS WERE		NOTES*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
8*b*	*S. decimina*	....	NaOH, 0.01¶	....	2	G
9	*S. longata*	Platinum, 96.0	....	....	1	E, for 9 days D, after 22 days
10	*Do.*	Platinum, 48.0	Tap water, 50.0	....	1	G
11*a*	*Do.*	*do.*	NaOH, 0.01‡	....	1	E
11*b*	*S. decimina*	*do.*	*do.*	1	....	E; heavy sheaths within 17 hours, colored brown by Pt.
12*a*	*S. longata*	Platinum, 86.4	NaOH, 0.02¶	1	1	G; few slight sheaths in one culture
12*b*	*S. decimina*	*do.*	*do.*	1	....	E; heavy sheaths within 17 hours; colored by Pt.
13*a*	*S. longata*	*do.*	NaOH, 0.01¶	....	2	E
13*b*	*S. decimina*	*do.*	*do.*	2	....	E; heavy sheaths within 17 hours, colored by Pt.
14	*S. longata*	Silver, 90, and various lower concentrations	....	....	19	Mostly D, in some cases G
15*a*	*Do.*	Silver, 0.135	NaOH, 0.02‡	....	1	D
15*b*	*Do.*	Silver, 0.09	*do.*	....	1	Mostly D
15*c*	*Do.*	Silver, 0.045	*do.*	....	1	Gp
16	*Do.*	Platinum, 96.0; silver, various concentrations below 90.0	....	....	5	E
17	*Do.*	Silver, various concentrations below 90.0	Salts to form Crone's solution, 0.5	....	5	E

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* The general condition of the filaments is here denoted by the symbols E, G, etc., as in previous tables.

† All *Spirogyra longata* used was from the stock culture unless otherwise noted.

‡ Sodium hydrate solution employed in the preparation of the colloidal gold solution.

§ The *Spirogyra decimina* was newly brought from the open.

|| The *Oscillatoria* was used from a culture in a 0.5 per cent Sachs's solution thickened with 1 per cent agar.

¶ Sodium hydrate solution as used in preparation of colloidal gold solution, but prepared at another time.

either species of *Spirogyra* in a 0.01 per cent solution of $AuCl_2$ which contained also 0.02 per cent or 0.01 per cent NaOH (V, 3, 4). When filaments of *S. longata* were subjected to the action of the sodium hydrate of the gold solution (approximately 0.02 per cent concentration of NaOH), in the absence of the colloidal gold, no swellings were developed (V, 5). Diluting the sodium hydrate solution by adding an equal volume of non-toxic water was without effect (V, 6*a*). *S. decimina*, however, produced some colorless sheaths on the second day in this 0.01 per cent solution of NaOH, though the majority of the filaments were by this time dead. No sheaths were evident on the first day (V, 6*b*). In sodium hydrate solutions with concentrations of 0.02 and 0.01 per cent, respectively, prepared at another time and perhaps not exactly similar to the solution in which the colloidal gold was formed, neither species of the alga exhibited any swellings (V, 7, 8).

With colloidal platinum no sheaths were formed by *Spirogyra longata* in the undiluted solution (V, 9), nor were they evident in this solution diluted with an equal volume of tap water (V, 10) or with an equal volume of a 0.02 per cent solution of NaOH (V, 11*a*). In this alkaline platinum solution *S. decimina*, on the contrary, formed heavy sheaths within 7 hours, these being colored a deep brown by the platinum (V, 11*b*). Similarly *S. longata* gave only slight sheaths on but a few filaments in slightly diluted platinum solution containing 0.02 per cent of NaOH; it failed to produce any sheaths at all in platinum solution to which 0.01 per cent of NaOH had been added (V, 13*a*). *S. decimina* formed heavy brown sheaths in both of the last named solutions (V, 12*b*, 13*b*).

No sheaths appeared upon *Spirogyra longata* in any strength of silver solution here tested, either with (V, 15) or without (V, 14) NaOH. Nor were sheaths formed by this species in any strength of silver to which was added the undiluted platinum solution (V, 16) or inorganic salts to form 0.5 per cent Crone's solution (V, 17). In these solutions the filaments remained in excellent condition throughout the experiments.

When no swelling became apparent, as in the presence of colloidal platinum alone or in that of colloidal platinum and colloidal silver together, the cell walls remained uncolored, and seemed to be unaffected by the colloids; but when swelling occurred in the outer portions of the cell walls, as in a mixture of gold or platinum with NaOH, the swollen portions became deeply colored. In these cases the appearance of the colored wall was similar to that produced by the action of a soluble stain like Bismarck brown, a feature which suggests specific adsorption of the suspensoid as the cause of this color change. BREDIG (**2**) and ZSIGMONDY (**16**) mention similar instances of the staining of fungi by colloidal gold. In the former case this was not taken into the filaments but was deposited on the external surfaces of the walls. In the latter case the gold continued to be absorbed until the solution became entirely decolorized.

Apparently the only other published work on the effect of colloidal metals on plants other than bacteria is that of GALEOTTI (**6**). This author carefully studied the effect of colloidal solutions of copper on a species of *Spirogyra* and compared with these the effect of $CuSO_4$ solutions computed to contain the same amounts of copper in the ionic condition. GALEOTTI found colloidal copper poisonous at lower concentrations than was ionic copper, although the action of the former was less rapid. The author concluded that ionic copper produced a rapid effect by combining with the protoplasm, while the colloidal metal, at least in lower concentrations, was slowly active as a catalyser, accelerating certain breaking-down processes within the cells. Addition of 0.01 per cent of NaCl to higher concentrations of colloidal copper rendered these somewhat less toxic and almost entirely corrected the toxicity of the colloid in lower concentrations. The toxicity of ionic copper, on the other hand, was not modified in the presence of 0.01 per cent of NaCl. The influence of the chloride in diminishing the toxic effect of colloidal copper was attributed to some change induced in the colloid itself.

Following GALEOTTI, it may be suggested that the diminution in the toxicity of the lower concentrations of colloidal silver, brought about in the present studies by the inorganic salts of Crone's solution, may have been due to some action of the salts upon the colloid. The effect of animal charcoal and of colloidal platinum upon the toxicity of colloidal silver, that of colloidal platinum upon the toxicity of toxic waters and salt solutions, and that of colloidal platinum, gold, and silver upon the toxicity of NaOH may be considered as probably due to surface phenomena such as adsorption. It appears from the experiments that ionic gold and ionic platinum ($AuCl_2$ and $PtCl_4$) exerted no influence upon the toxicity of NaOH and $MgSO_4$, respectively. The information at hand is not sufficient to warrant any suggestion as to why the transfer of filaments from colloidal gold solution to non-toxic distilled water was so quickly fatal to *Spirogyra*. A possibly similar case, in which the toxicity of a solution was decreased by addition of a colloid, has been reported by HATCHER (7), who found that the toxicity of strychnine injected hypodermically into frogs and guinea-pigs was considerably decreased when the alkaloid was administered in a solution of gum acacia. In this connection it needs only to be suggested that the finely divided and otherwise highly absorbent solids which, as has been shown by various authors since the time of Nägeli (NÄGELI 14, TRUE and OGLEVEE 15, BREAZEALE 1, LIVINGSTON *et al.* 11, LIVINGSTON 12, JENSEN 9, HOYT 8), exert a correcting influence upon many toxic solutions, partake of some of the properties of colloids. One essential feature, in this regard, of all such solids, as well as of both suspensoids and emulsoids which exert such corrective influence, seems to be great extension of surface.

It is interesting to notice that, although colloidal platinum produced improvement in ordinary distilled water, a weak solution of agar, when added to such water, produced no improvement. It has also been pointed out in another paper (8) that a 1 per cent agar hydrogel made with this water was found to be only slightly toxic, while a similar 2 per cent agar hydrogel was decidedly injurious. Evidence is not at hand for any attempt to interpret these phenomena critically.

The results obtained furnish no indication as to what physicochemical properties may be ascribed the extreme toxicity of colloidal silver and copper when colloidal gold and platinum are so slightly injurious. These results agree with those obtained by FOÀ and AGGAZZOTTI (5), who showed that, although colloidal platinum, gold, and silver were about equally effective in hindering the development of bacteria, colloidal platinum and gold in strengths of

125 ppm. were fatal to only two kinds of the bacteria tested, while finely divided colloidal silver containing 70 ppm. was fatal to all the bacteria used in their investigations. MORSE (13), from some preliminary experiments on several unicellular animals and plants, concluded that colloidal platinum and gutta percha are without appreciable effect on protoplasm. It should be noted that, in the present investigation, alga filaments in colloidal solutions often showed injury suddenly, after they had remained for several days in apparently excellent or good condition.

The swelling of cell walls caused by akaline colloidal gold or platinum, as observed in these studies, seemed to depend upon the presence of both the colloid and the alkali, since the walls seemed unaffected by the colloid alone and were very slightly altered by a solution of NaOH alone, while marked swelling was produced by a mixture of colloid and NaOH. A mixture of $AuCl_2$ and NaOH was without effect in this connection. The failure of colloidal silver to induce swelling may possibly be associated with those properties of this colloid that produced its toxic effect upon the protoplasm. That no sheaths were formed in those alkaline solutions of silver that permitted the alga to live in apparently good condition may be related to the extreme dilution of these silver solutions.

Results strikingly similar to those obtained in the present investigation from the use of colloidal gold and platinum with NaOH were obtained by KLEBS (10) upon precipitating various substances (as calcium phosphate, copper sulphide, iron ferrocyanide, alizarine-lead oxide) within the gelatinous sheaths of several species of *Zygnema* and other algae. Under these conditions there were formed colored, swollen, gelatinous masses, which separated from the cells in a way quite like that described above. This swelling occurred not only on the walls of living cells, but also on the walls of cells killed by ether vapor or weak alcohol, although not on the walls of those killed by corrosive sublimate, lead acetate, or some other substances. Not all precipitates caused swelling, and the appearance of the gelatinous masses, when formed, differed with different precipitates and different species of algae.

Since the formation of these swollen masses was caused only by precipitates composed of small particles, while it was caused by widely different chemical substances, and furthermore, since substances deposited as crystals within the sheaths were without effect in this respect, KLEBS concluded that the swelling did not depend on the chemical nature of the precipitated substance, but did depend on the size of the particles composing the precipitate. He was led by these and other considerations to the view that the presence of solid particles between the particles of the gelatinous sheath was the mechanical cause of processes which ended in the breaking-off of the jelly along with the precipitate. He described this result as being brought about by two steps: first, the particles which were precipitated within the sheath slipped out, surrounded by portions of the jelly, and gathered at the periphery, where they were cemented together by the jelly into a layer surrounding each filament; second, this layer containing the precipitated particles was raised up in irregular forms and thrown off by the swelling of the jelly. He concluded that the swelling was not due to a reaction of the protoplasm to a stimulus, that substances which affected the protoplasm also affected the structure of the jelly and consequently its capacity for swelling, but that the observed phenomena were not then explainable in their molecular-physical relation.

On grounds of physical chemistry, as has already been suggested, it seems possible to bring such phenomena as those observed by KLEBS and those of the present studies into the same category with many cases of enzymatic catalysis. Disintegration of cell walls in plants, related to enzyme action, is frequently accompanied by such alterations in the material of the walls as to increase the extent of their imbibing power, so that they swell much more than is usual and assume a gelatinous or mucilaginous consistency. That some colloidal metals (as platinum) may exert influences upon organic material, that are quite similar in many respects to the effects of enzymes, is now generally held. Furthermore, the acidity or alkalinity of the medium employed is well known to influence greatly enzymatic catalysis, some enzymes being more effective in an acid and others in an alkaline solution. That enzymes are characteristically colloid and that their activity is closely related to this fact seems also to be established by the work of many biochemists. The possibility is suggested, therefore, that the disintegration effects above described, produced in alkaline solutions of colloidal metals, may be considered as catalytic phenomena due to the colloids, the latter acting in a manner more or less similar to that in which the organic colloidal catalytes which we term enzymes are known to operate; if catalysis results frequently from enormously extended surfaces, it may not be without the bounds of probability to suppose that alterations in organic material, similar to those produced by the organic enzymes, may be brought about by inorganic colloids such as those dealt with in the present paper. It seems also possible to relate the swollen cell walls observed by KLEBS, in the study above discussed, to some catalytic action which has altered the water-imbibing power of the wall material, rather than directly to the forces which are active in producing absorption and formation of his precipitates. To attempt the extension of this general hypothesis to the details of the various instances would be out of place at the present time.

It is worthy of note that the swellings with which this paper has had to do were most pronounced in *Spirogyra decimina*, less so in *S. longata* from the stock culture, and were not exhibited at all by the latter form from the culture in Crone's solution. That the last mentioned material was the most vigorous, while the stock material of *S. longata* was next in order, and the material of *S. decimina* was apparently least healthy, suggests that the portion of the cell wall taking part in the swelling differed in the three sorts of filaments; susceptibility to the swelling influence exerted by the colloidal metals seems to have been greater as the alga was less vigorous. Whatever may have been its explanation, we have here another example illustrating the fact that similar filaments of the same algal species, but cultivated under different conditions, may be expected to attain different physiological states, thus becoming physiologically quite dissimilar. Such differences are apparently indicated in the present instance by the diversity above noted in the susceptibility of the outer portions of the cell walls in the three kinds of algal material. The existence of different physiological states in individuals of a single species is of course well known to all experimenters. It has been previously shown for *Spirogyra*, by the present writer (**8**), by parallel and exactly similar experiments carried out with plants from the same original stock but kept for some time under different cultural conditions. In experiments designed to test the effect of special environmental conditions upon organisms it is absolutely necessary to guard, as far as may be possible, against these different physiological states in the material employed. Such a statement is self-evident, yet many results and criticisms found in physiological literature seem to have been brought into existence through failure to take this fundamental condition sufficiently into account.

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Summary

1. Colloidal silver was fatal to filaments of *Spirogyra* in all concentrations above 0.045 ppm. and was injurious in concentrations as low as 0.00225 ppm. The weaker solutions of silver were rendered almost or entirely non-toxic, during the period of the experiments, by addition of colloidal platinum, animal charcoal, or inorganic salts to form a 0.5 per cent Crone's solution.

2. A solution containing 90 ppm. of colloidal gold and approximately 0.02 per cent of NaOH was only very slightly injurious.

3. A solution containing 96 ppm. of colloidal platinum was almost non-injurious during the period of the experiments, and, in less concentrated solutions, partially corrected the toxicity of tap water, ordinary distilled water, and solutions of KCl, $MgSO_4$, and colloidal silver.

4. Colloidal gold, colloidal platinum, and, to a less extent, colloidal silver, in low concentration, all partially prevented injury to the alga filaments by toxic solutions of NaOH. Addition of $AuCl_2$ to a toxic solution of NaOH, or of $PtCl_4$ to a toxic solution of $MgSO_4$ did not render the hydrate solutions less toxic.

5. When *Spirogyra* was placed in a solution containing colloidal platinum or colloidal gold together with NaOH, the outer portions of the cell walls swelled, forming crumpled, gelatinous sheaths

which became deeply stained by the metal. This swelling was especially pronounced when the filaments were transferred from the alkaline colloidal gold or platinum solution to non-toxic distilled water. The swollen masses thus produced often parted from the rest of the wall, leaving the latter uncolored and apparently unaffected.

6. The cell walls were apparently unaffected by colloidal silver, either alone or with NaOH, or with salts to form a 0.5 per cent Crone's solution. They seemed to be unaffected by colloidal platinum alone or by a mixture of this with colloidal silver. Only very slight swelling of the walls occurred in solutions of NaOH alone. Marked swelling occurred only with the solution of colloidal gold or of colloidal platinum in the presence of NaOH. A solution of $AuCl_2$ and NaOH was without effect in this regard.

7. Filaments of *Spirogyra* originally from the same culture, but grown for a time in different media, exhibited different reactions in the solutions of colloidal gold and NaOH, as well as in the other toxic solutions here employed.

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